

**METHODS OF ANALYSIS BY THE U.S. GEOLOGICAL SURVEY
NATIONAL WATER QUALITY LABORATORY--
DETERMINATION OF CHLORINATED PESTICIDES IN AQUATIC
TISSUE BY CAPILLARY-COLUMN GAS CHROMATOGRAPHY WITH
ELECTRON-CAPTURE DETECTION**

By Thomas J. Leiker, James E. Madsen, Jeffrey R. Deacon,
and William T. Foreman

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CONVERSION FACTORS, ABBREVIATED WATER-QUALITY UNITS, AND ADDITIONAL ABBREVIATIONS AND SYMBOLS

Multiply	By	To obtain
centimeter (cm)	3.94×10^{-1}	inch
gram (g)	3.52×10^{-2}	ounce
liter (L)	0.265	gallon
meter (m)	3.28	foot
microliter (μ L)	2.64×10^{-7}	gallon
milligram (mg)	3.53×10^{-5}	ounce
milliliter (mL)	2.64×10^{-4}	gallon
millimeter (mm)	3.94×10^{-2}	inch
nanometer (nm)	3.93×10^{-8}	inch

Degree Celsius ($^{\circ}$ C) may be converted to degree Fahrenheit ($^{\circ}$ F) by using the following equation:

$$^{\circ}\text{F} = 9/5 (^{\circ}\text{C}) + 32.$$

Abbreviated water-quality units used in this report are as follows:

$^{\circ}$ C/min	degree Celsius per minute	ng/ μ L	nanogram per microliter
μ g/kg	microgram per kilogram	pg/ μ L	picogram per microliter
mg/mL	milligram per milliliter	lb/in ²	pounds per square inch
mL/min	milliliter per minute		

Other abbreviations used in this report:

CAS	Chemical Abstract Service
CCV	continuing calibration verification
GC	gas chromatography
GC/ECD	gas chromatography/electron capture detection
GPC	gel permeation chromatography
id	inside diameter
K-D	Kuderna-Danish concentrator
MDL	method detection limit
NAWQA	National Water-Quality Assessment program
NIST	National Institute of Standards and Technology
NWQL	National Water Quality Laboratory
PCB	polychlorinated biphenyls
PEM	performance evaluation mix
QC	quality control
RSD	relative standard deviation
SOP	standard operating procedure
SRM	Standard Reference Material
USEPA	U.S. Environmental Protection Agency
USFWS	U.S. Fish and Wildlife Service

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ABSTRACT

A method for the determination of chlorinated organic compounds in aquatic tissue by dual capillary-column gas chromatography with electron-capture detection is described. Whole-body-fish or corbicula tissue is homogenized, Soxhlet extracted, lipid removed by gel permeation chromatography, and fractionated using alumina/silica adsorption chromatography. The extracts are analyzed by dissimilar capillary-column gas chromatography with electron-capture detection. The method reporting limits are 5 micrograms per kilogram ($\mu\text{g/kg}$) for chlorinated compounds, 50 $\mu\text{g/kg}$ for polychlorinated biphenyls, and 200 $\mu\text{g/kg}$ for toxaphene.

INTRODUCTION

Historically the water quality of ground, surface, or impounded water is often established by determining the concentration of organic compounds present in a 1-L sample. Because of surface-water variability in time and ground-water availability in space, an analysis based on a 1-L sample might not produce a true water-quality picture. Hydrophobic organic compounds such as chlorinated pesticides, PCBs, and other chlorinated organic compounds often are present in the water column at concentrations less than the detection limits of conventional analytical techniques. These sparingly soluble compounds usually exhibit high octanol to water partition coefficients ($\text{Log } K_{\text{OW}} > 4$) and are selectively partitioned from the water column into organic material associated with bed and suspended sediment and into the lipid tissue of stream biota.

The water quality of a riverine system is based on, but not limited to, atmospheric deposition, ground water, agricultural runoff, municipal and industrial discharges, and barge transportation of chemical, food, and agricultural products along major rivers. Runoff from forest, cattle, pork, and poultry industries also has an impact on water quality. Various studies have determined contaminant levels in sediment, water, and aquatic tissue as an index of water quality and agricultural run-off patterns (Barthel and others, 1969; Laska and others, 1976; Sabourin and others, 1984; Winger and Andreasen, 1985; Leiker and others, 1991). Other studies

have demonstrated that organic contaminants with high Log K_{ow} , which are present in the water column at nanogram-per-liter levels, will bioconcentrate within the lipid tissues of aquatic biota (Jaffe and others, 1985; Oliver and Niimi, 1985; Pereira and others, 1988; Swackhamer and Hites, 1988; Leiker and others, 1991). Because of these studies, measuring the pesticide content in tissue samples might provide data to determine long-term water-quality trends, identify sources of contamination in rivers and streams, monitor changes in land-use patterns, and indicate when new hydrophobic pesticides are being used.

This report describes a method for determining chlorinated pesticides and industrial chemicals in whole-body-fish and corbicula tissue. The method was developed by the U.S. Geological Survey for its National Water-Quality Assessment (NAWQA) program for use at the National Water Quality Laboratory (NWQL). Other methods of the U.S. Geological Survey for determination of organic compounds are described by Wershaw and others (1987). The method was implemented at the NWQL in November 1992.

This report provides detailed information regarding sample preparation and analysis, interferences, calculations, accuracy and precision of the method validation, explanation of quality-control data, round-robin results for further validation, and method detection limits for 28 chlorinated compounds.

The authors acknowledge the technical support of Brooke Connor, Dennis Markovchick, Tammy Thompson, Gene Slocum, and Anthony Sofia. Additional thanks are accorded to Stuart McKenzie and Stephen Porter, who supplied tissue samples for the method's development and validation; Wilfred Pereira, Charles Demas, and Greg Foster provided helpful discussions.

ANALYTICAL METHOD

Organic Compounds and Parameter Codes:

**Chlorinated pesticides, recoverable from aquatic tissue,
capillary-column gas chromatography, electron-capture detection,
O-9125-94 (see table 1)**

1. Scope and application

1.1 This method is suitable for the determination of selected organochlorine-containing pesticides and industrial chemicals in whole-body fish and corbicula tissue at concentrations of 5 $\mu\text{g}/\text{kg}$ for chlorinated pesticides, 50 $\mu\text{g}/\text{kg}$ for PCBs, and 200 $\mu\text{g}/\text{kg}$ for toxaphene. The method is applicable for those hydrophobic compounds that exhibit $\text{Log } K_{ow} > 4$. The method is used to determine the concentration of 28 chlorinated organic compounds listed in table 1. These 28 compounds were selected for analysis to fulfill the requirements of the NAWQA

program. In addition, 3,5-dichlorobiphenyl and *alpha*-HCH-d₆ were used as surrogates for quality control (table 1). Recovery from a matrix spike might not indicate the true concentration of a compound originally present in the sample because of mechanisms that are only active in a living organism that might irreversibly bind chlorinated pesticides to the tissues.

Table 1. Compounds, codes, and Chemical Abstract Service registry numbers

[WATSTORE, Water Data Storage and Retrieval System; NWQL, National Water Quality Laboratory; CAS, Chemical Abstract Service; --, no code assigned]

Compound	WATSTORE code	NWQL code	CAS registry number
Aldrin	49353	7030	309-00-2
<i>cis</i> -Chlordane	49380	7001	5103-71-9
<i>trans</i> -Chlordane	49379	7002	5103-74-2
DCPA (dacthal)	49378	7003	1861-32-1
<i>o,p'</i> -DDD	49374	7007	53-19-0
<i>p,p'</i> -DDD	49375	7006	72-54-8
<i>o,p'</i> -DDE	49373	7008	3424-82-6
<i>p,p'</i> -DDE	49372	7009	72-55-9
<i>o,p'</i> -DDT	49377	7004	789-02-6
<i>p,p'</i> -DDT	49376	7005	50-29-3
Dieldrin	49371	7010	60-57-1
Endrin	49370	7011	72-20-8
<i>alpha</i> -HCH	49366	7016	319-84-6
<i>beta</i> -HCH	49365	7017	319-85-7
<i>delta</i> -HCH	49364	7018	319-86-8
<i>gamma</i> -HCH (lindane)	49363	7019	58-89-9
Heptachlor	49369	7012	76-44-8
Heptachlor epoxide	49368	7013	1024-57-3
Hexachlorobenzene	49367	7014	118-74-1
<i>o,p'</i> -Methoxychlor	49362	7020	30667-99-3
<i>p,p'</i> -Methoxychlor	49361	7021	72-43-5
Mirex	49360	7022	2385-85-5
<i>cis</i> -Nonachlor	49359	7023	5103-73-1
<i>trans</i> -Nonachlor	49358	7024	39765-80-5
Oxychlordane	49357	7025	27304-13-8
Pentachloroanisole	49356	7027	1825-21-4
Total PCB	49354	7029	--
Toxaphene	49355	7028	8001-35-2
3,5-Dichlorobiphenyl (surrogate)	49264	7035	34883-41-5
<i>alpha</i> -HCH-d ₆ (surrogate)	49261	7034	--

1.2 Method performance was validated by analyzing seven replicates at low and high concentrations of all 28 selected compounds in the following sample matrices: whole-body-fish tissue, corbicula tissue, and reagent blank consisting of sodium sulfate. Further validation involved analysis of Standard Reference Material (SRM-1588 cod liver oil) from the National Institute of Standards and Technology (NIST), and U.S. Environmental Protection Agency (USEPA) Quality Control Fish Tissue. U.S. Fish and Wildlife Service round-robin samples, from its contract laboratories program, were also analyzed.

2. Summary of method

2.1 Whole-body-fish or corbicula tissue is homogenized into a single composite.

2.2 A 10-g sample aliquot of homogenized tissue is thoroughly mixed with 100 g of granular anhydrous sodium sulfate and Soxhlet extracted overnight with methylene chloride.

2.3 After extraction, the extract is concentrated to a volume of 5.0 mL. A 1-mL aliquot is removed for percentage lipid determination. A 2-mL aliquot of the extract is injected into a gel permeation chromatograph (GPC) to separate the lipid material and other interferences from the method compounds.

2.4 After the compounds have been collected from the GPC, the extract is solvent exchanged into hexane, separated into two fractions on a column packed from top to bottom with 1 cm of sodium sulfate, 5 g of 8.5 percent water-deactivated alumina, 3 g of 2 percent water-deactivated silica, and 0.5 cm of sodium sulfate.

2.4.1 The first fraction contains the PCBs, DDE, and other nonpolar organics.

2.4.2 The second fraction contains toxaphene, chlordane components, DDT, DDD, and other more polar organic compounds.

2.5 Each fraction is concentrated to a volume of 1 mL and analyzed by dual capillary-column gas chromatography with electron-capture detection.

3. Interferences

Compounds recovered from a sample matrix, which have chemical and physical properties that are similar to but are not chromatographically resolved from the compounds of interest, can interfere.

4. Apparatus and equipment

4.1 Blender, Waring.

- 4.2 Soxhlet extractors.
 - 4.2.1 Round-bottom flasks, 500-mL.
 - 4.2.2 Condensers.
- 4.3 Soxhlet thimble, glass-fiber, precleaned overnight at 450°C.
- 4.4 Boiling chips (large).
- 4.5 Kuderna-Danish (K-D) apparatus.
 - 4.5.1 Flasks, 500-mL K-D.
 - 4.5.2 Three-ball Snyder columns.
 - 4.5.3 Receivers, 10-, 25-, and 50-mL K-D.
- 4.6 GPC autosampler vials, 4-mL.
- 4.7 Miscellaneous items.
 - 4.7.1 Evaporative concentrators.
 - 4.7.2 Metal spatulas.
- 4.8 Analytical balance.
 - 4.8.1 Balance capable of accurately weighing up to 1,200 g ± 0.1 g.
 - 4.8.2 Analytical balance capable of accurately weighing 200 g ± 0.1 mg.
- 4.9 Gas chromatograph equipped with the following items:
 - 4.9.1 Capillary column: two 25-m x 0.25-mm inside diameter (id) fused-silica-capillary columns--one is coated with 5 percent diphenyl and 95 percent dimethyl polysiloxane (Restek Rtx-5), and the other is coated with 14 percent cyanopropyl and 86 percent dimethyl polysiloxane (Restek Rtx-1701) or equivalent.
 - 4.9.2 Autosampler capable of variable syringe draw-up rate.
 - 4.9.3 Detectors, two nickel-63 electron-capture.
 - 4.9.4 Data station capable of producing chromatograms and quantitative reports.
 - 4.9.5 Temperature-controlled injection ports, detectors, and gas chromatography (GC) oven capable of multitemperature program ramps.

4.10 Gel permeation chromatograph equipped with the following items:

4.10.1 Solvent delivery system with variable pumping rates.

4.10.2 Autosampler with sample storage carousel and variable syringe draw rate.

4.10.3 Gel permeation columns: two Waters Envirogel GPC Prep Columns 19 x 150 mm and 19 x 300 mm or equivalent.

4.10.4 Ultraviolet detector set at 254 nm.

4.10.5 Fraction collector.

4.10.6 Data station or integrator capable of producing chromatograms and quantitative reports.

4.11 Mason jars, pint- and quart-size, precleaned overnight at 450°C.

5. Reagents and consumable materials

5.1 Helium gas chromatography (GC) carrier gas (grade 5).

5.2 Nitrogen GC makeup gas (grade 5).

5.3 Nitrogen, ultrapure gas for evaporation.

5.4 Scrubbers to remove oxygen and impurities from carrier gas.

5.5 Solvents: Acetone, hexane, isooctane, methylene chloride, methanol, toluene, cyclohexane; B&J brand, ultrapure pesticide quality or equivalent.

5.6 Syringes, 10-, 25-, 50-, and 100-μL.

5.7 Class A pipet, 2-mL.

5.8 Disposable 127- and 229-mm Pasteur pipets, precleaned by baking at 450°C overnight.

5.9 Sodium sulfate, granular, preclean by baking at 450°C overnight.

5.10 Obtain standards for calibration curves, surrogate and internal standards, retention-time markers, continuing calibration verification (CCV) and performance evaluation mix (PEM) standards, and reagent spike solutions at a certified concentration or prepare from pure primary standards.

5.10.1 Calibration standards include all method compounds at concentrations of 5, 10, 20, 50, 100, and 200 picograms per microliter (pg/ μ L). Prepare the standards in hexane or cyclohexane. The PCB calibration standard contains a 1:1:1 mixture of Aroclor 1242, 1254, and 1260 at 200 pg/ μ L each in hexane or cyclohexane. The toxaphene calibration standard is 1,200 pg/ μ L.

5.10.2 Add surrogate standards consisting of 3,5-dichlorobiphenyl and *alpha*-HCH-d₆, in hexane, at a concentration of 2.5 ng/ μ L to each sample prior to Soxhlet extraction. Use a 100- μ L syringe to fortify the sample with 100 μ L of surrogate spiking solution. Alternate surrogate compounds may be used after demonstrating adequate performance. This standard provides sample processing information on every environmental sample that is analyzed.

5.10.3 Add retention-time-marker solution of tetrachloro-*m*-xylene and decachlorobiphenyl to the sample at the time the sample extract is transferred to a vial and sealed. Prepare the retention-time-marker solution at 10 ng/ μ L in hexane, and fortify the sample with 10 μ L of this solution. Use this retention-time marker to monitor and correct for small drift in GC retention time of method compounds. Also use it for internal standard quantification when required. The conditions for internal standard quantification are dependent on the stability of the instrument and the sample matrix. Use internal standard quantification only when external quantification fails because of matrix effects or instrumental instability (McNair and Bonelli, 1969; U.S. Environmental Protection Agency, 1990).

5.10.4 The CCV standards include all method compounds prepared at 50 pg/ μ L. Analyze this standard after every fifth environmental sample to monitor and ensure the validity of the calibration curve throughout the GC analysis for all samples and compounds.

5.10.5 The PEM standards include *alpha*-, *beta*-, and *gamma*-HCH (10 pg/ μ L), *p,p'*-DDT (100 pg/ μ L), endrin (50 pg/ μ L), and *p,p'*-methoxychlor (250 pg/ μ L). Use these standards to monitor the resolution of the chromatographic separations, and chromatographic and injection port degradation of method compounds, thereby providing data to indicate when to perform maintenance on the chromatographic system.

5.10.6 Prepare a 1.25-ng/ μ L reagent spike solution, in hexane, consisting of all single-component method compounds, and spike 100 μ L directly into the sodium sulfate prior to Soxhlet extraction.

5.10.7 Prepare a GPC system performance verification standard, in methylene chloride, to monitor the performance of the GPC. Use this standard to monitor the retention times and resolution of the GPC columns that separate the method compounds from the coextracted lipid material. The standard consists of corn oil, 63,000 mg/L; *bis*-2-ethyl-hexylphthalate, 2,000 mg/L; perylene, 45 mg/L; and sulfur, 220 mg/L.

5.11 Prepare working standards from pure primary materials as follows: Using an analytical balance capable of weighing to ± 0.1 mg, weigh 5 to 10 mg of primary standard directly into a 5- or 10-mL volumetric flask. Dilute to volume with hexane or cyclohexane. Verify that all of the solid material has gone into solution. After preparation, transfer the standard to an appropriate vial labeled with the date that the standard was prepared, concentration, solvent, purity of the primary material, and a mark on the side of the vial to record the volume at time of preparation. Seal the vial with a Teflon-lined screw cap and store in a freezer for a maximum of 6 months. Date and re-mark the level of solvent every time an aliquot is withdrawn.

5.12 Prepare or obtain working standard solutions at concentrations from 1 to 2 mg/mL. Prepare all subsequent dilutions with 10-, 25-, 50-, or 100- μ L syringes. Transfer the aliquot required to prepare a standard at a given concentration directly into a volumetric flask and dilute to volume with hexane or cyclohexane. Transfer the solution to a Teflon-lined screw-cap vial and store for a maximum of 3 months.

5.13 After preparation, check all standard stock solutions against existing standards. All standard stock solutions must fall within 20 percent of existing standards. Under no circumstances are these solutions to be validated separately or on different days. Never store the standard stock solutions in volumetric flasks or at ambient temperature overnight, regardless of the storage container.

6. Sample collection and preparation

6.1 Collect fish and corbicula samples according to guidelines set forth by Crawford and Luoma (1993). Briefly, collect fish samples by electroshocking, wrap in aluminum foil, freeze, and ship frozen to NWQL. Collect corbicula samples, depurate for 22 hours, wrap in aluminum foil, and ship frozen to NWQL.

6.2 Preparation and homogenization of whole-body fish tissue.

6.2.1 Allow samples, wrapped in aluminum foil, to thaw overnight at ambient temperature.

6.2.2 After thawing, composite and thoroughly homogenize all fish (typically 5 to 10) from a specific sampling site by five repetitive processings through a meat grinder. Place about 300 g of homogenized tissue in a clean jar and store the homogenized tissue in a freezer. Discard the remaining bulk quantity of tissue. Observe all safety requirements established by the manufacturer of the meat grinder.

6.2.3 Wash all components of the meat grinder with soap and hot water, and rinse with organic-free distilled water, methanol or acetone, and methylene chloride between samples.

6.3. Preparation and homogenization of corbicula.

6.3.1 After thawing, remove the corbicula tissue from the shell using precleaned spatulas that have been baked at 450°C overnight. Open the shell with the spatula and scrape the tissue from the shell directly into a clean mason jar. Composite and homogenize all tissue from a specific sampling site. Wear gloves to prevent transfer of finger oil to the sample.

6.3.2 Homogenize the corbicula tissue with a stainless steel blender. Place up to 300 g of homogenized tissue in a clean jar and store the homogenized tissue in a freezer. Clean the components of the blender that come in contact with the sample in the same manner as the meat grinder (see section 6.2.3).

6.4 After homogenizing composite samples, weigh about 10 g of homogenized tissue directly into a preweighed pint-size mason jar that contains 100 g of granular anhydrous sodium sulfate. Weigh the jar to the nearest 0.1 g and record the actual weight of sample that is placed in the mason jar. Refreeze the sample sodium sulfate mixture and store frozen until the sample is prepared for extraction.

6.5 Prior to extraction, while the sample is still frozen in the mason jar, use a blender to thoroughly homogenize the sample sodium sulfate mixture to a free-flowing powder. If any of the sample sodium sulfate mixture is retained on the blender blades, scrape the material or rinse the blades into the mason jar containing the sample sodium sulfate mixture.

6.6 After homogenization, quantitatively transfer the sample sodium sulfate mixture to a glass-fiber Soxhlet thimble that was baked at 450°C overnight. Add 100 µL of a 2.5-ng/µL surrogate standard solution to the sample prior to extraction. Rinse the mason jar three times with 20 mL of methylene chloride. Add the rinses to the Soxhlet apparatus, and extract the sample for a minimum of 8 hours with 250 mL of methylene chloride. The solvent should cycle through the Soxhlet apparatus about every 15 or 20 minutes.

6.7 After extraction, add 10 g of granular anhydrous sodium sulfate to the round-bottom flask that contains the extract to remove any water that may be present. Allow the extract and granular anhydrous sodium sulfate to set for 1 hour. Then decant the extract into an assembled K-D unit (500-mL flask with three-ball Synder column). Rinse the sides of the round-bottom flask containing the sample extract and granular anhydrous sodium sulfate three times with 20 mL of methylene chloride, and add the rinses to the K-D unit. Concentrate the extract and rinses by K-D to a volume of 5 mL. Filter the extract through a disposable 127-mm Pasteur pipet that contains 25 mm of granular anhydrous sodium sulfate, collect the filtered extract in a 10-mL K-D receiver, rinse the sodium sulfate column with two column volumes of methylene chloride, and combine the rinses with the sample extract. Concentrate the extract to 5.0 mL under a gentle stream of nitrogen at ambient temperature.

6.8 Transfer a 1-mL aliquot of the extract to a tared 15-mL culture tube for percentage lipid determination.

6.8.1 Evaporate the 1 mL of extract to dryness at ambient temperature and under a gentle stream of nitrogen, or use a turbovap.

6.8.2 After removing the solvent, weigh the culture tube until a constant weight is achieved and record the constant weight.

6.8.3 Calculate the percentage of lipid using the following formula:

$$\text{Percentage lipid} = \frac{(W_{ts} - W_t)}{1 \text{ mL}} \times \frac{5 \text{ mL}}{S_w} \times 100 \quad (1)$$

where W_{ts} = W_t plus the weight of lipid contained in 1 mL of extract after solvent has been removed;

W_t = culture tube tare weight; and

S_w = weight of sample extracted (use weight recorded in section 6.4).

6.9 Transfer the remaining 4 mL of extract (S_v) to a tared 4-mL GPC vial, seal with the Teflon-lined screw cap, and pressurize with nitrogen.

6.9.1 Pressurize each GPC vial with 30 lb/in² of nitrogen for 30 seconds by piercing the septum with the pressurization needle. Withdraw the syringe needle from the septum. Rinse the needle by bubbling into a few milliliters of methylene chloride between each sample. Weigh and record the weight of the filled vial (V_w) to the nearest 1 mg. (CAUTION: Do not place the needle into the extract.)

6.10 Place the sample vial into the carousel of the GPC autosampler. Inject a 2-mL aliquot of the extract into the GPC for separation of lipid material from the method compounds. Collect the GPC fraction containing the method compounds into a 50-mL K-D receiver tube. For details regarding the setup, operation, and compound collection times, refer to SOP OT0028.0 (unpublished SOPs are available from the NWQL). After the sample has been injected into the GPC, reweigh the vial with the Teflon-lined screw cap and record its weight to the nearest 0.1 mg (V_w'). Calculate the equivalent mass of tissue extract injected into the GPC, using the following formula:

$$S_v = (V_w - V_w')/1.32 \quad (2)$$

where S_v = the volume of extract, in milliliters, that is injected into the GPC;

V_w = the weight of the vial plus sample prior to GPC;

V_w' = the weight of the vial minus the sample that has been injected into the GPC; and

1.32 = density of methylene chloride, in grams per milliliter.

Then calculate the equivalent mass of tissue extract injected into the GPC, using

$$S_a = (S_w/5 \text{ mL}) * S_v \quad (3)$$

where S_a = the equivalent mass of tissue extract injected into the GPC; and
 S_w = weight of sample extracted in grams (use weight recorded in section 6.4).

Use this value to calculate the final concentrations of compound, in micrograms per kilogram, in the sample.

6.10.1 Use a Waters liquid chromatographic system to carry out the gel permeation chromatography. Lipid material is separated from the compounds of interest using two Waters Envirogel GPC Prep Columns. The first column is a guard column 19 x 150 mm. The second column is 19 x 300 mm. The mobile phase is methylene chloride, and the flow rate of the mobile phase is 4.5 mL/min.

6.10.2 The separation of coextracted lipid material from method compounds is monitored by an ultraviolet detector set at a wavelength of 254 nm. If the retention times shift by more than 2 minutes, as determined by the GPC evaluation standard, terminate the sequence and take corrective action.

6.10.3 The following is a typical GPC sequence for standards, environmental samples, and quality-control (QC) samples: GPC evaluation standard, seven environmental samples, duplicate environmental sample, four environmental samples, SRM, reagent spike, reagent blank, and GPC evaluation standard.

6.11 Collect the compounds of interest in a 50-mL receiver. After collection, attach a three-ball Snyder column to the 50-mL receiver, add boiling chips, place the unit on a steam bath, and concentrate to a volume of 5 mL. Remove the unit from the steambath, and allow to cool. Then add 25 mL of hexane and new boiling chips to the extract, and concentrate the extract to a volume of 5 mL on a steam bath. Further concentrate the extract to 1 mL under a gentle stream of nitrogen at ambient temperature. At this point all methylene chloride has been removed from the extract. If the methylene chloride is not removed, the separations in the next step will fail.

6.12 Separate the extract into two fractions using a 265-mm by 12.5-mm id chromatographic column equipped with a 75-mL reservoir and porous frit. Dry pack the column from top to bottom with 1 cm of granular sodium sulfate, 5 g of 8.5 percent water-deactivated neutral alumina (initially activated at 135°C), 3 g of 2 percent water-deactivated silica (initially activated at 135°C), and 0.5 cm of granular sodium sulfate.

6.12.1 Prerinse the column with 50 mL of hexane.

6.12.2 As the hexane rinse sinks into the top of the sodium sulfate, transfer the 1 mL of sample extract to the top of the sodium sulfate, taking care not to disturb the alumina silica bed, and elute with 30 mL of hexane (discard the first 5 mL of hexane), collecting this first eluant in a 25-mL K-D receiver. This first fraction contains PCBs, *p,p'*-DDE, HCB, and other nonpolar organic compounds. Just prior to adding 25 mL of 50 percent (volume:volume) acetone in hexane, place a fresh 25-mL K-D receiver into position to collect the second fraction. The second fraction contains toxaphene, chlordane components, DDT, DDD, HCH isomers, and other more polar organic compounds.

6.13 Concentrate each fraction to a volume of 1 mL, label a 2-mL GC autosampler vial, transfer extract to the GC autosampler vial, add exactly 10 μ L of a 10-ng/ μ L retention-time-marker solution to the extract, cap with a Teflon-lined cap, and store in a freezer until the sample is analyzed.

7. Analysis of sample extracts

7.1 Analyze sample extracts by dual capillary-column gas chromatography with electron-capture detection. Make chromatographic separations with two dissimilar 30-m by 0.25-mm id capillary columns, one of which is coated with 5 percent diphenyl and 95 percent dimethyl polysiloxane, and the other is coated with 14 percent cyanopropylphenyl and 86 percent dimethyl polysiloxane.

7.2 Hold the GC oven temperature at 50°C for 1 minute and program at 15°C/min to 140°C, then program at 1°C/min to a temperature of 220°C; next program the oven at 4°C/min to a temperature of 280°C and hold at the upper temperature for 20 minutes. Hold the injection port temperature at 220°C and the detector temperature at a minimum of 300°C. Other GC temperature programs are permissible as long as acceptable chromatographic separations, compound identifications, and quantitations are maintained.

7.3 Place the vials containing the extracts into the autosampler tray.

7.4 Set the autosampler to rinse syringe five times with clean solvent prior to the 2- μ L injection of sample extract.

7.5 Base the compound identifications on comparison of GC retention times with authentic standards. Base the quantitation on a six-point calibration curve for all chlorinated pesticides and a single-point calibration for PCBs and toxaphene. Produce the six-point calibration curve for the chlorinated pesticides at the beginning of the analysis for each set of environmental samples and associated quality-control samples. The calibration range for all single-component compounds is 5, 10, 20, 50, 100, and 200 pg/ μ L. The calibration curve is acceptable if the correlation coefficient is greater than 0.995. The recommended calibration standard for PCB is 600 pg/ μ L (a 1:1:1 mixture of Aroclor 1242, 1254, and 1260 at 200 pg/ μ L

for each Aroclor type), and toxaphene is 800 pg/μL. Depending on the concentration of PCB and toxaphene in the sample extract, the analyst has the discretion to alter the amount of standard to be more representative of the concentrations in the actual sample extract.

7.6 As part of the instrumental quality-control program, analyze continuing calibration verification (CCV) standards at 50 pg/μL and performance evaluation mix (PEM, section 5.10.5) standards consisting of *alpha*-, *beta*-, and *gamma*-HCH, *p,p'*-DDT, and *p,p'*-methoxychlor after every fifth environmental sample. Analyze the CCV standard to ensure that the calibration has not drifted more than 30 percent from the expected value. Analyze the PEM standard to monitor chromatographic resolution, sensitivity, and degradation, primarily for *p,p'*-DDT and endrin breakdown during injection. If the breakdown of *p,p'*-DDT or endrin exceeds 30 percent when analyzing fraction-2 (this fraction contains endrin, *o,p'*- and *p,p'*-DDT, *o,p'*- and *p,p'*-DDD among other more polar organic compounds), then terminate the analysis, perform preventive maintenance, and re-analyze the extracts. If the CCV has drifted more than 30 percent, refer to SOP OT0022.0 for guidance (unpublished SOPs are available from the NWQL).

7.7 Report the compound concentration data from the column that produces the lowest concentration, unless it is documented through calibration standards, CCV, or PEM that a specific compound on a specific capillary column is not performing adequately because of compound coelution, degradation, or interference.

7.8 The following compounds are known to coelute on the specified capillary column:

7.8.1 Known coelutions on Rtx-5: *o,p'*-DDT and *p,p'*-DDD;
Heptachlor epoxide and oxychlordane.

7.8.2 Known coelutions on Rtx-1701: *cis*-Nonachlor and *p,p'*-DDD;
Oxychlordane and DCPA.

7.9 The following is a typical GC analytical sequence for standards, environmental samples, and quality-control samples:

Hexane wash

PEM

5 pg/μL calibration standard

10 pg/μL calibration standard

20 pg/μL calibration standard

50 pg/μL calibration standard

100 pg/μL calibration standard

200 pg/μL calibration standard

600 pg/μL PCB calibration standard

800 pg/μL toxaphene calibration standard

Method blank
Method spike
SRM
Two environmental samples
CCV
PEM
Five environmental samples
CCV
PEM
Five environmental samples
CCV
PEM
Five environmental samples
CCV
PEM

7.10 Compound identification is confirmed if the compound is detected at the expected retention time on both GC columns. The degree of error associated with the retention time is matrix and compound dependent. The allowable retention-time error is based on the average of three retention times of standards from the initial calibration GC sequence. The quantitative value reported is column dependent.

8. Calibration

Compounds are calibrated (and subsequently quantitated in samples) by using results obtained on both capillary columns.

8.1 *Multipoint external standard calibration for single-component compounds.*

Option: The internal standard method of compound calibration and quantitation uses either tetrachloro-*m*-xylene or decachlorobiphenyl, provided that there are no chromatographic interferences with these compounds. Details of internal standard quantitation are not provided here. In the external standard method described below, tetrachloro-*m*-xylene or decachlorobiphenyl is used as a retention-time marker to assist in compound identification.

8.1.1 For single-component compounds, calibrate using multipoint curves produced from analysis of the 5 to 200 pg/μL (or other) calibration standards. Regress the peak area of the compound in the standard solution (A_C) in relation to the mass (in picograms) of the compound in the standard injected using the following simple linear model:

$$A_c = m \times (C_c \times V_1) + b \quad (4)$$

where m = compound-specific slope, in area per picograms;
 C_c = concentration of the compound in the standard, in picograms per microliter;
 V_1 = volume of calibration standard injected into GC/ECD, in microliters; and
 b = compound-specific y -intercept, in area.

NOTE: Other regression models may be used as appropriate.

8.1.2 For compounds that exhibit coelutions on both analytical columns (for example, p,p' -DDD), calibrate by using one or more separate standards that contain only one of the coeluting compounds. For example, use separate standards that contain p,p' -DDD but not coeluting o,p' -DDT (on Rtx-5), and not coeluting *cis*-nonachlor (on Rtx-1701). Identification and quantification of compounds that coelute on both columns requires careful consideration by the analyst. For example, p,p' -DDD can be quantified on the Rtx-1701 column if there are no other chlordane components present in the sample (thus suggesting no coeluting *cis*-nonachlor). In most cases, the compound that coelutes on both columns will need to be reported as an upper limit value, or not reported because of coeluting interference.

8.2 *External standard calibration for PCBs and toxaphene.* For PCBs and toxaphene, an overall response factor is computed by summing the peak areas for 10 to 15 representative congeners (selected on the basis of adequate peak intensity and separation from other congener, target compound, and interferent peaks) and dividing by the concentration of the PCB or toxaphene standard. For PCBs, a 1:1:1 mixture of Aroclor 1242, 1254, and 1260 at 200 pg/ μ L each (or 600 pg/ μ L total concentration) was typically used as the calibration standard. The response factor is calculated by equation 5:

$$RF = \frac{\text{Sum of selected congener peak areas in standard}}{C_m \times V_1} \quad (5)$$

where RF = response factor, in area per picograms;
 C_m = total PCB or toxaphene concentration in standard, in picograms per microliter; and
 V_1 = volume of standard injected into GC/ECD, in microliters.

An average response factor is computed if multilevel calibration standards are used for PCBs and toxaphene.

9. Calculations

9.1 Calculate the concentration of compounds in the sample extract. For the individual compounds, use the compound-specific regression parameters (equation 4) from the calibration curve to calculate the raw amount of compound in the sample extract:

$$RA = \frac{(A_s - b)}{m \times V_2} \quad (6)$$

where RA = raw amount of compound in sample extract, in picograms per microliter;
 A_s = the peak area of the identified component in the sample extract;
 b = compound-specific y -intercept, in area;
 m = compound-specific slope, in area per picograms; and
 V_2 = final volume of extract injected into GC/ECD, in microliters.

9.2 Calculate the concentration of PCBs and toxaphene in the sample. Sum the peak areas for the 10 to 15 PCB or toxaphene congeners in the sample that match the retention times of those peaks selected for the PCB or toxaphene calibration standards. Calculate the raw amount of PCBs or toxaphene in the sample extract, as follows:

$$RA_m = \frac{\text{Sum of selected congener peak areas in sample}}{RF \times V_2} \quad (7)$$

where RA_m = raw amount of PCB or toxaphene in sample extract, in picograms per microliter;
 RF = the PCB or toxaphene response factor, in area per picograms (calculated from equation 5); and
 V_2 = final volume of extract injected into GC/ECD, in microliters.

9.3 Calculate the concentration (C_s) of the identified compound in the sample, in micrograms per kilogram of wet-weight tissue, using

$$C_s = \frac{RA \times V_3}{S_a} \quad (8)$$

where C_s = concentration of compound in sample, in micrograms per kilogram (nanograms per gram);
 RA = raw amount of compound, in nanograms per milliliter (picograms per microliter) (calculated from equation 6);
 V_3 = final volume of extract just prior to GC/ECD, in milliliters; and
 S_a = equivalent weight of tissue injected into the GPC (calculated from equation 3).

NOTE: For PCBs and toxaphene, substitute RA_m from equation 7 for RA in equation 8.

9.4 Calculate the percent recovery of the surrogate compounds in each sample, using

$$R_a = \frac{C_s}{(C_a \times V_a)/S_w} \times 100 \quad (9)$$

where R_a = recovery of surrogate in sample, in percent;
 C_s = concentration of surrogate in sample, in nanograms per gram (= micrograms per kilogram) (calculated from equation 8);
 C_a = concentration of compound in the surrogate standard added to the sample, in nanograms per microliter;
 V_a = volume of surrogate standard added to the sample, in microliters; and
 S_w = weight of sample extracted in grams (use weight recorded in section 6.4).

9.5 Calculate the percent recovery of compounds in reagent spike sample, using

$$R_b = \frac{C_s}{(C_b \times V_b)/S_w} \times 100 \quad (10)$$

where R_b = recovery of spiked compound in the reagent spike sample, in percent;
 C_s = concentration of compound in reagent spike sample, in nanograms per gram (micrograms per kilogram) (calculated from equation 8);
 C_b = concentration of compound in organochlorine spike standard added to sample, in nanograms per microliter;
 V_b = volume of reagent spike standard added to the sample, in microliters; and
 S_w = weight of sample extracted in grams (use weight recorded in section 6.4).

9.6 Calculate the percent recovery of compounds in SRM sample, using

$$R_{SRM} = \frac{C_s}{C_{srm}} \times 100 \quad (11)$$

where R_{SRM} = recovery of compound in the SRM sample, in percent;
 C_s = concentration of compound in SRM sample, in nanograms per gram (micrograms per kilogram) (calculated from equation 8); and
 C_{srm} = certified concentration of compound in the SRM sample, in nanograms per gram.

9.7 Calculate the percent breakdown of *p,p'*-DDT and endrin on the GC/ECD from injections of the PEM using the following equations:

$$\text{Percent } p,p'\text{-DDT breakdown} = \frac{A_{p,p'\text{-DDE}} + A_{p,p'\text{-DDD}}}{A_{p,p'\text{-DDT}} + A_{p,p'\text{-DDD}} + A_{p,p'\text{-DDE}}} \times 100 \quad (12)$$

and

$$\text{Percent endrin breakdown} = \frac{A_{\text{endrin aldehyde}} + A_{\text{endrin ketone}}}{A_{\text{endrin}} + A_{\text{endrin aldehyde}} + A_{\text{endrin ketone}}} \times 100 \quad (13)$$

where A_{compound} = peak area of given compound in the PEM chromatogram.

9.8 Compute the CCV percent difference.

9.8.1 Calculate the raw amount for each compound in the CCV standard (RA_{ccv}) using equation 6.

9.8.2 Calculate the percent difference between the determined and expected CCV concentrations, using

$$\text{CCV percent difference} = \frac{RA_{ccv} - C_e}{C_e} \times 100 \quad (14)$$

where RA_{ccv} = calculated raw amount of compound in CCV standard, in picograms per microliter; and
 C_e = expected concentration of compound in CCV standard, in picograms per microliter.

10. Reporting of results

Report concentrations of compounds as follows: less than 10 µg/kg, two significant figures; 10 to 1,000 µg/kg, three significant figures. Report results less than 5.0 µg/kg as "less than method reporting limit."

DISCUSSION OF RESULTS

Method Performance

Method performance was evaluated by analyzing samples of nonspiked homogenized whole-body fish tissue, homogenized corbicula tissue, as well as samples of the same tissues spiked at two different concentrations. Each sample was evaluated using seven replicates. In addition, seven replicates at two concentrations of a reagent spike were analyzed. Seven replicates of NIST SRM-1588 cod liver oil were analyzed as part of the evaluation of method accuracy. To further validate the analytical method, two samples from the U.S. Fish and Wildlife Service round-robin evaluation were analyzed in duplicate, and a USEPA quality-control sample was analyzed in quadruplicate.

The method performance data from nonmatrix spikes, matrix spikes, and SRM analyses are listed in the Supplement, tables 2 through 12. The compounds are listed in order of detection on Rtx-5.

Because of the high background levels of some of the method compounds in the sample that was selected for the 30- and 100- $\mu\text{g}/\text{kg}$ matrix spike, a second set of experiments was conducted on a separate sample spiked at 40 $\mu\text{g}/\text{kg}$ to produce performance data at a low concentration. Unfortunately, the second sample selected also had high concentrations of selected method compounds that precluded establishing performance data at this level. No further attempt was made to locate a sample with suitable background concentrations and repeat the recovery experiments because of the length of time required to produce method performance data.

The performance data presented in tables 2 through 16 (see Supplement) indicate that this method will provide accurate and precise concentration data for all method compounds. However, some method compounds may present problems in analysis; an example of a problem is listed in table 4. The concentration data for *o,p'*- and *p,p'*-DDD have percent RSDs (relative standard deviations) that are greater than 60 percent. The concentration data for *o,p'*- and *p,p'*-DDD are lower in determinations A and B than for determinations C, D, E, and F. Initially this limited data set indicates that a problem exists with the samples. If this were the case, all concentration data for all method compounds would have percent RSDs that are comparable to those of the *o,p'*- and *p,p'*-DDD. With the exception of these two compounds, the precision of the method compounds is acceptable (percent RSD < 23 percent).

There are two possibilities that would explain the data. First, there are unknown compounds that are coeluting with *o,p'*- and *p,p'*-DDD in determinations C, D, E, and F that account for their higher concentrations. Second, *o,p'*- and *p,p'*-DDT are susceptible to thermal degradation and may thermally degrade to *o,p'*- and *p,p'*-DDD inside the GC injection port when it is contaminated. These data indicate that thermal degradation indeed is occurring. In determinations A and B, *o,p'*- and *p,p'*-DDT are not thermally degrading to *o,p'*- and *p,p'*-DDD (table 4). Higher reported concentrations of *o,p'*- and *p,p'*-DDD in determinations C, D, E, and F

indicate that *o,p'*- and *p,p'*-DDT are thermally degrading to their respective DDD components upon injection of the sample extract. This line of reasoning can be applied to table 12, which lists percent recovery from the NIST SRM-1588. The performance evaluation mix was not analyzed during the method-performance phase of this project. This type of problem is identified and corrected by examining the results of the PEM standard that is analyzed after every fifth environmental sample.

To further validate this tissue methodology, the NWQL participated in several round-robin studies sponsored by the U.S. Fish and Wildlife Service and the USEPA. The samples from the U.S. Fish and Wildlife service were homogenized fish tissue. The samples from the USEPA were freeze-dried and mixed with sodium sulfate. The results from other laboratories that participated in the Fish and Wildlife Service round-robin study and the results produced by NWQL using this method are listed in tables 13a and 13b (see Supplement). The study consisted of two homogenized fish-tissue samples that were analyzed for the following compounds: *alpha*- and *gamma*-HCH, hexachlorobenzene, heptachlor epoxide, oxychlordane, *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, *o,p'*- and *p,p'*-DDE, *o,p'*- and *p,p'*-DDD, *o,p'*- and *p,p'*-DDT, dieldrin, endrin, mirex, total PCBs, and toxaphene. On the basis of a duplicate concentration for each sample, the data produced by NWQL from the U.S. Fish and Wildlife samples fall within one standard deviation of the mean for all method compounds. When duplicates are not averaged but are considered as individual analyses, about 80 percent of all data fell within one standard deviation, and 100 percent of the data fell within two standard deviations. The calculated range, provided by the USFWS, is based on the individual analyses from all participating laboratories.

The results of the USEPA quality-control samples are listed in table 14 (see Supplement). Four replicate samples contained *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, oxychlordane, total chlordane (defined as the sum of *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane), *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, total DDX (defined as the sum of *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT), and percentage of lipid. The total chlordane and DDX values are within the acceptable range as determined by the USEPA. The precision of the total chlordane and DDX measurements is within one standard deviation. Although the average percentage of lipid is outside the acceptable range, this value is directly dependent on the solvent used for extraction. The samples analyzed by NWQL were extracted by methylene chloride. The reported values were from a hexane extraction.

Method Detection Limits

Method detection limits were estimated with data from the 2.5-μg/kg reagent spikes and homogenized fish tissue that were fortified at the 2.0-μg/kg level. Matrix based method detection limits were determined on a fish homogenate chosen from submitted samples that contained low levels of contaminants. The method detection limits listed in tables 15 and 16 (see Supplement) are single calibration MDLs

determined in two matrices. These detection limits are not used as method reporting limits. They neither account for the range of lipid composition, lipid concentration, and variety of aquatic tissues that are routinely analyzed, nor the day-to-day variation in instrument performance. The method detection limits were determined according to the U.S. Environmental Protection Agency (1992, p. 565-567).

QUALITY ASSURANCE

Process the samples and analyze in a set consisting of 16 samples--12 environmental samples and 4 quality-control samples. The quality-control samples consist of a sodium sulfate blank, a reagent spike, SRM, and a duplicate environmental sample. Add a surrogate spike to all samples, including QC samples, prior to extraction. The amount to be added is established in paragraph 5.10.2. Add retention-time markers to all samples, including QC samples, prior to GC analysis. The amount to be added is established in paragraph 5.10.3.

Interpretation of Laboratory Quality-Control Data

The percentage of surrogate recovery is intended to provide data on the overall performance of the analytical method as it relates to a specific sample. Environmental data is not to be corrected for surrogate recoveries. If any portion of the method fails prior to fractionation on the alumina silica column, the failure will be reflected in low recoveries of both surrogates. If the surrogate recovery is within three sigma of the average surrogate recovery (the control limits), the method is within analytical control for that sample. If the recoveries of both surrogates from both GC columns are greater than three sigma of the average surrogate recovery, this result might indicate coeluting interferences with the surrogate or an unknown matrix effect. This result might not bias the reported concentrations of the method compound, but the chromatogram needs to be reviewed thoroughly. If the percentage of recovery of both surrogates falls below the lower three-sigma control limit, the preparation for that sample is considered out of analytical control. As a result, the sample will need to be reextracted and reanalyzed. If the surrogate from a single fraction is less than 30 percent recovery, the extract is to be reanalyzed.

Reagent spike recovery data are used to monitor the overall performance of the analytical method. The reagent spike percent recoveries are not influenced by matrix effects. Reagent spike recoveries outside of three sigma might indicate that the analytical data for the entire set are not acceptable, and the set needs to be reextracted and reanalyzed.

To further evaluate method performance, the analyst examines the SRM and surrogate recoveries of all samples in the set. If the reagent spike recoveries, SRM, and surrogate recoveries are not within established guidelines, reevaluate the entire

set and reextract and reanalyze. If the reagent spike, SRM, and surrogate recoveries are within established guidelines, the analytical data are considered to be acceptable. The surrogate and reagent spike data are never used to correct environmental data.

CONCLUSIONS

On the basis of data presented, this analytical method can be used routinely for the determination of chlorinated pesticides in whole-body-fish and corbicula tissue at low microgram-per-kilogram concentrations. At present (1995), method reporting limits are set at 5 µg/kg wet weight for chlorinated compounds, 50 µg/kg for polychlorinated biphenyls, and 200 µg/kg for toxaphene. Reporting limits are subject to change depending on background interferences, limitation in sample size, matrix effects, or high levels of other compounds, such as PCBs in the sample extract.

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SUPPLEMENT: METHOD PERFORMANCE DATA

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Table 2. Concentration data of nonspiked whole-body fish tissue used for 40-microgram-per-kilogram spike

[µg/kg, micrograms per kilogram; Std. dev., standard deviation; RSD, relative standard deviation; <, less than]

Compound	Concentration units (µg/kg wet weight)							Mean	Std. dev.	RSD (percent)
	A	B	C	D	E	F	G			
<i>alpha</i> -HCH	4.8	8.6	10.2	11.6	11.4	9.3	6.8	9.0	2.5	27.7
Hexachlorobenzene	<5	<5	<5	<5	<5	<5	<5			
Pentachloroanisole	<5	<5	<5	<5	<5	<5	<5			
<i>beta</i> -HCH	3.3	4.9	4.9	5.0	4.7	3.9	3.0	4.2	.8	19.7
<i>gamma</i> -HCH	5.5	7.3	6.7	8.0	6.9	5.5	4.4	6.3	1.2	19.6
<i>delta</i> -HCH	22.1	27.6	30.9	41.1	37.3	29.0	27.3	30.8	6.4	21.0
Heptachlor	<5	<5	<5	<5	<5	<5	<5			
Aldrin	<5	<5	<5	<5	<5	<5	<5			
DCPA	4.8	11.6	10.8	8.9	9.8	8.9	6.8	8.8	2.4	26.7
Heptachlor epoxide	<5	<5	<5	<5	<5	<5	<5			
Oxychlordane	<5	<5	<5	<5	<5	<5	<5			
<i>trans</i> -Chlordane	<5	<5	<5	<5	<5	<5	<5			
<i>o,p'</i> -DDE	<5	<5	<5	<5	<5	<5	<5			
<i>cis</i> -Chlordane	19.5	24.1	27.9	37.9	33.2	27.3	22.5	27.5	6.3	23.1
<i>trans</i> -Nonachlor	17.0	14.5	17.6	17.0	17.3	19.0	11.3	16.2	2.5	15.7
Dieldrin	128	124	135	138	137	141	91	128	17.1	13.4
<i>p,p'</i> -DDE	1,267	880	862	831	846	847	876	916	156	17.0
<i>o,p'</i> -DDD	33.1	31.2	37.4	40.7	34.1	35.1	22.8	33.5	5.6	16.8
Endrin	9.5	18.2	11.5	24.0	14.1	13.5	11.3	14.6	5.0	34.4
<i>cis</i> -Nonachlor	5.5	6.0	6.9	6.7	7.2	7.2	4.6	6.3	1.0	15.9
<i>p,p'</i> -DDD	335	308	338	336	360	362	246	326	39.9	12.2
<i>o,p'</i> -DDT	<5	<5	<5	<5	<5	<5	<5			
<i>p,p'</i> -DDT	199	181	186	176	153	172	123	170	25.1	14.7
<i>o,p'</i> -Methoxychlor	<5	<5	<5	<5	<5	<5	<5			
<i>p,p'</i> -Methoxychlor	<5	<5	<5	<5	<5	<5	<5			
Mirex	<5	<5	<5	<5	<5	<5	<5			

Table 3. Percent recovery of 40-microgram-per-kilogram spike in homogenized whole-body fish tissue

[Std. dev., standard deviation; RSD, relative standard deviation; ND, not detected; NS, not spiked, standards not available; NR, not reported, background concentrations greater than spike]

Compound	Recovery of individual compounds, in percent							Mean	Std. dev.	RSD (percent)
	A	B	C	D	E	F	G			
<i>alpha</i> -HCH	ND	ND	ND	ND	ND	ND	ND			
Hexachlorobenzene	87	88	94	92	106	97	99	95	7	7
Pentachloroanisole	NS	NS	NS	NS	NS	NS	NS			
<i>beta</i> -HCH	ND	ND	ND	ND	ND	ND	ND			
<i>gamma</i> -HCH	52	64	90	22	33	20	25	44	26	60
<i>delta</i> -HCH	ND	ND	ND	ND	ND	ND	ND			
Heptachlor	89	91	92	89	102	92	96	93	5	5
Aldrin	61	65	78	76	88	90	61	74	12	16
DCPA	73	88	125	132	149	136	150	122	30	25
Heptachlor epoxide	76	92	126	119	149	137	150	121	28	23
Oxychlorthane	NS	NS	NS	NS	NS	NS	NS			
<i>trans</i> -Chlordane	61	65	68	66	74	74	76	69	6	8
<i>o,p'</i> -DDE	NR	NR	NR	NR	NR	NR	NR			
<i>cis</i> -Chlordane	67	78	82	76	90	88	89	81	8	10
<i>trans</i> -Nonachlor	60	76	83	82	82	83	95	80	11	13
Dieldrin	NR	NR	NR	NR	NR	NR	NR			
<i>p,p'</i> -DDE	NR	NR	NR	NR	NR	NR	NR			
<i>o,p'</i> -DDD	31	41	48	49	62	59	67	51	13	25
Endrin	89	93	98	94	88	97	91	93	4	4
<i>cis</i> -Nonachlor	68	78	78	74	87	97	91	82	10	12
<i>p,p'</i> -DDD	NR	NR	NR	NR	NR	NR	NR			
<i>o,p'</i> -DDT	NR	NR	NR	NR	NR	NR	NR			
<i>p,p'</i> -DDT	NR	NR	NR	NR	NR	NR	NR			
<i>o,p'</i> -Methoxychlor	94	108	100	102	94	85	76	94	11	11
<i>p,p'</i> -Methoxychlor	101	119	137	127	137	132	143	128	14	11
Mirex	100	108	108	105	120	109	111	109	6	6
Lipid	11.8	9.7	9.2	10.3	9.0	10.6	9.6	10	1	10

Table 4. Concentration data of nonspiked whole-body fish tissue used for 30- and 100-microgram-per-kilogram spike

[$\mu\text{g}/\text{kg}$, micrograms per kilogram; Std. dev., standard deviation; RSD, relative standard deviation; <, less than]

Compound	Concentration units ($\mu\text{g}/\text{kg}$ wet weight)					Mean	Std. dev.	RSD (percent)
	A	B	C	D	E			
<i>alpha</i> -HCH	<5	<5	<5	<5	<5			
Hexachlorobenzene	5	4.8	5.3	4.9	6.3	5.1	0.7	14
Pentachloroanisole	<5	<5	<5	<5	<5			
<i>beta</i> -HCH	2.4	7.5	7	6.8	8.2	6.2	2.1	34
<i>gamma</i> -HCH	<5	<5	<5	<5	<5			
<i>delta</i> -HCH	<5	<5	<5	<5	<5			
Heptachlor	<5	<5	<5	<5	<5			
Aldrin	<5	<5	<5	<5	<5			
DCPA	7.4	4.7	4.7	6.7	6	6.2	1.3	21
Heptachlor epoxide	11.2	11.4	10.9	10.6	14.1	11.3	1.5	13
Oxychlorthane	<5	<5	<5	<5	<5			
<i>trans</i> -Chlordane	6.3	5.8	7.2	5.8	8.1			
<i>o,p'</i> -DDE	11.2	11.1	10.6	9.5	10.9	10.5	.8	7.6
<i>cis</i> -Chlordane	18.2	17	16.9	16.9	21.6	17.7	2.1	12
<i>trans</i> -Nonachlor	36.5	36.4	32.8	34.6	42.4	35.4	4.2	12
Dieldrin	162	161	114	116	130	130	27	21
<i>p,p'</i> -DDE	1,470	1,583	894	1,282	1,293	1,240	281	23
<i>o,p'</i> -DDD	8.1	7.2	48	47.9	59.3	35.5	22.3	63
Endrin	16.6	15.3	19.5	8.1	16.3	14.4	4.2	29
<i>cis</i> -Nonachlor	10.7	9.8	11.6	8.7	13.4	10.6	1.8	17
<i>p,p'</i> -DDD	36	35	405	411	508	293	205	70
<i>o,p'</i> -DDT	18.6	15.7	21.5	21.1	26.6	20.2	3.8	19
<i>p,p'</i> -DDT	331	317	274	291	366	306	40	13
<i>o,p'</i> -Methoxychlor	<5	<5	<5	<5	<5			
<i>p,p'</i> -Methoxychlor	<5	<5	<5	<5	<5			
Mirex	<5	<5	<5	<5	<5			

Table 5. Percent recovery of 30-microgram-per-kilogram spike in homogenized whole-body fish tissue

[Std. dev., standard deviation; RSD, relative standard deviation; ND, not detected;
NS, not spiked, standards not available; NR, not reported, background concentrations greater than spike]

Compound	Recovery of individual compounds, in percent							Mean	Std. dev.	RSD (percent)
	A	B	C	D	E	F	G			
<i>alpha</i> -HCH*	98	98	<5	113	103	61	102	96	18	19
Hexachlorobenzene	89	93	93	108	109	67	107	95	15	16
Pentachloroanisole	NS	NS	NS	NS	NS	NS	NS			
<i>beta</i> -HCH	88	62	62	88	93	59	92	78	16	20
<i>gamma</i> -HCH	82	88	88	107	34	80	34	73	28	39
<i>delta</i> -HCH	69	82	82	106	99	48	98	84	20	24
Heptachlor	92	92	92	103	112	65	110	95	16	17
Aldrin	78	76	76	90	91	52	90	79	14	18
DCPA*	117	90	ND	67	106	78	104	94	19	20
Heptachlor epoxide*	53	48	ND	61	65	23	66	53	16	31
Oxychlordanes	NS	NS	NS	NS	NS	NS	NS			
<i>trans</i> -Chlordane*	81	78	ND	93	95	53	82	80	15	19
<i>o,p'</i> -DDE	56	41	87	43	47	40	45	51	17	32
<i>cis</i> -Chlordane	95	94	94	111	121	45	119	97	26	27
<i>trans</i> -Nonachlor	105	108	108	131	143	86	140	117	21	18
Dieldrin	NR	NR	NR	NR	NR	NR	NR			
<i>p,p'</i> -DDE	NR	NR	NR	NR	NR	NR	NR			
<i>o,p'</i> -DDD	NR	NR	NR	NR	NR	NR	NR			
Endrin	68	68	68	94	119	62	117	85	25	29
<i>cis</i> -Nonachlor	29	27	27	34	40	17	39	30	8	26
<i>p,p'</i> -DDD	NR	NR	NR	NR	NR	NR	NR			
<i>o,p'</i> -DDT	84	84	84	96	115	61	113	91	19	21
<i>p,p'</i> -DDT	NR	NR	NR	NR	NR	NR	NR			
<i>o,p'</i> -Methoxychlor*	111	120	ND	149	140	109	137	127	17	13
<i>p,p'</i> -Methoxychlor	72	78	78	93	94	52	92	80	18	19
Mirex*	108	103	ND	116	118	75	116	106	16	15

*n=6; all others n=7.

Table 6. Percent recovery of 100-microgram-per-kilogram spike in homogenized whole-body fish tissue

[Std. dev., standard deviation; RSD, relative standard deviation; ND, not detected;
NR, not reported, background concentrations greater than spike]

Compound	Recovery of individual compounds, in percent						Mean	Std. dev.	RSD (percent)
	A	B	C	D	E	F			
<i>alpha</i> -HCH	100	103	102	104	106	87	100	7	7
Hexachlorobenzene	79	77	86	81	88	66	80	8	10
Pentachloroanisole	97	95	103	98	104	80	96	9	9
<i>beta</i> -HCH	141	80	160	83	89	66	103	38	37
<i>gamma</i> -HCH	80	87	83	92	93	70	84	8	10
<i>delta</i> -HCH	90	98	94	104	104	82	95	8	9
Heptachlor	80	86	84	84	84	70	81	6	7
Aldrin	96	93	86	99	96	81	92	7	8
DCPA*	108	111	118	108	97	ND	108	8	7
Heptachlor epoxide	80	86	84	84	84	70	81	6	7
Oxychlorthane	96	93	86	99	96	81	92	7	8
<i>trans</i> -Chlordane*	108	111	118	108	97	ND	108	8	7
<i>o,p'</i> -DDE*	29	24	31	40	39	ND	33	7	20
<i>cis</i> -Chlordane*	72	70	74	81	89	ND	77	8	10
<i>trans</i> -Nonachlor*	96	55	88	73	77	ND	78	16	20
Dieldrin*	86	105	122	102	118	ND	107	14	13
<i>p,p'</i> -DDE	NR	NR	NR	NR	NR	NR			
<i>o,p'</i> -DDD*	93	103	104	102	98	ND	100	5	5
Endrin*	99	107	89	119	95	ND	102	11	11
<i>cis</i> -Nonachlor*	91	41	93	97	94	ND	83	24	28
<i>p,p'</i> -DDD	NR	NR	NR	NR	NR	NR			
<i>o,p'</i> -DDT	ND	ND	ND	ND	ND	ND			
<i>p,p'</i> -DDT	NR	NR	NR	NR	NR	NR			
<i>o,p'</i> -Methoxychlor*	93	72	84	80	82	ND	82	8	9
<i>p,p'</i> -Methoxychlor*	85	64	78	75	83	ND	77	8	11
Mirex*	89	76	79	80	80	ND	81	5	6
Lipid	40.2	34.6	32.5	33.9	28.0	37.4	34	4	12

*n=5.

Table 7. Concentration data of nonspiked corbicula tissue used for 5- and 40-microgram-per-kilogram spike

[µg/kg, micrograms per kilogram; Std. dev., standard deviation; RSD, relative standard deviation; <, less than]

Compound	Concentration units (µg/kg wet weight)							Mean	Std. dev.	RSD (percent)
	A	B	C	D	E	F	G			
<i>alpha</i> -HCH	<5	<5	<5	<5	<5	<5	<5			
Hexachlorobenzene	<5	<5	<5	<5	<5	<5	<5			
Pentachloroanisole	<5	<5	<5	<5	<5	<5	<5			
<i>beta</i> -HCH	<5	<5	<5	<5	<5	<5	<5			
<i>gamma</i> -HCH	<5	<5	<5	<5	<5	<5	<5			
<i>delta</i> -HCH	<5	<5	<5	<5	<5	<5	<5			
Heptachlor	<5	<5	<5	<5	<5	<5	<5			
Aldrin	<5	<5	<5	<5	<5	<5	<5			
DCPA	<5	<5	<5	<5	<5	<5	<5			
Heptachlor epoxide	<5	<5	<5	<5	<5	<5	<5			
Oxychlordane	<5	<5	<5	<5	<5	<5	<5			
<i>trans</i> -Chlordane	9.1	8.4	7.5	8.4	7.2	9.4	8	8.3	0.08	9.5
<i>o,p'</i> -DDE	<5	<5	<5	<5	<5	<5	<5			
<i>cis</i> -Chlordane	10.4	10.5	8.8	10.6	8.5	10.6	8.2	9.7	1.1	11.4
<i>trans</i> -Nonachlor	10.3	10.2	9.6	11	8.9	11.2	8.1	9.9	1.1	11.3
Dieldrin	14.1	14	12.4	13.7	10.5	14.1	10.3	12.7	1.7	13.2
<i>p,p'</i> -DDE	7.2	5.3	4.7	5	3.8	4.9	5.1	5.2	1	20.1
<i>o,p'</i> -DDD	<5	<5	<5	<5	<5	<5	<5			
Endrin	<5	<5	<5	<5	<5	<5	<5			
<i>cis</i> -Nonachlor	<5	<5	<5	<5	<5	<5	<5			
<i>p,p'</i> -DDD	9.9	9.5	9.2	10.7	7.4	10.6	8	9.3	1.2	13.4
<i>o,p'</i> -DDT	<5	<5	<5	<5	<5	<5	<5			
<i>p,p'</i> -DDT	<5	<5	<5	<5	<5	<5	<5			
<i>o,p'</i> -Methoxychlor	<5	<5	<5	<5	<5	<5	<5			
<i>p,p'</i> -Methoxychlor	<5	<5	<5	<5	<5	<5	<5			
Mirex	<5	<5	<5	<5	<5	<5	<5			
Lipid	2.3	2.3	2	2.2	2.1	2.4	2.1	2.2	0.1	6.4

Table 8. Percent recovery of 5-microgram-per-kilogram spike in homogenized corbicula tissue

[Std. dev., standard deviation; RSD, relative standard deviation; ND, not detected; NR, not reported]

Compound	Recovery of individual compounds, in percent							Mean	Std. dev.	RSD (percent)
	A	B	C	D	E	F	G			
<i>alpha</i> -HCH	70	81	72	107	87	85	78	83	12	15
Hexachlorobenzene	72	63	76	90	84	81	82	78	9	11
Pentachloroanisole	76	72	85	103	78	91	80	78	9	11
<i>beta</i> -HCH	86	80	91	101	93	100	89	91	7	8
<i>gamma</i> -HCH	65	67	87	150	136	131	107	106	34	32
<i>delta</i> -HCH	62	31	35	81	76	76	74	62	21	33
Heptachlor	71	59	74	84	76	72	70	72	7	10
Aldrin	65	56	68	83	75	72	73	70	8	12
DCPA	84	93	97	188	230	186	99	140	60	43
Heptachlor epoxide	ND	ND	ND	ND	ND	ND	ND			
Oxychlordane	118	108	124	153	139	141	144	132	16	12
<i>trans</i> -Chlordane	92	79	104	138	132	127	116	113	22	19
<i>o,p'</i> -DDE	14	14	15	20	26	21	20	19	4	24
<i>cis</i> -Chlordane	75	56	87	165	133	124	106	107	37	35
<i>trans</i> -Nonachlor	115	100	132	182	141	142	42	122	44	36
Dieldrin*	96	ND	119	194	151	158	146	144	34	24
<i>p,p'</i> -DDE	100	71	102	139	104	99	79	99	22	22
<i>o,p'</i> -DDD	77	68	64	104	82	95	81	82	14	17
Endrin	36	41	56	83	68	66	65	59	16	28
<i>cis</i> -Nonachlor	78	67	83	103	88	87	91	85	11	13
<i>p,p'</i> -DDD	NR	NR	NR	NR	NR	NR	NR			
<i>o,p'</i> -DDT	ND	ND	ND	ND	ND	ND	ND			
<i>p,p'</i> -DDT	105	77	104	151	123	181	123	123	34	28
<i>o,p'</i> -Methoxychlor	84	81	72	74	71	74	90	78	7	9
<i>p,p'</i> -Methoxychlor	95	92	108	113	111	47	48	88	29	33
Mirex	94	66	92	84	80	78	79	82	9	12

*n=6, all others n=7.

Table 9. Percent recovery of 40-microgram-per-kilogram spike in homogenized corbicula tissue

[Std. dev., standard deviation; RSD, relative standard deviation; ND, not detected]

Compound	Recovery of individual compounds, in percent										Mean	Std. dev.	RSD (percent)
	A	B	C	D	E	F	G						
<i>alpha</i> -HCH	ND	ND	ND	ND	ND	ND	ND						
Hexachlorobenzene	86	90	95	84	89	84	86			88	4	4	
Pentachloroanisole	79	82	87	78	85	82	83			82	3	4	
<i>beta</i> -HCH	81	86	91	84	91	84	91			87	4	5	
<i>gamma</i> -HCH	59	65	69	60	67	61	69			64	4	7	
<i>delta</i> -HCH	71	94	95	65	64	65	97			79	16	20	
Heptachlor	85	76	83	69	74	67	80			76	7	9	
Aldrin	88	77	77	63	64	55	78			72	11	16	
DCPA	73	79	85	76	85	78	81			80	4	6	
Heptachlor epoxide	88	89	95	82	92	90	89			89	4	4	
Oxychlorthane	ND	ND	ND	ND	ND	ND	ND						
<i>trans</i> -Chlordane	89	90	96	85	95	93	87			91	4	4	
<i>o,p'</i> -DDE	43	40	44	35	38	35	39			39	4	9	
<i>cis</i> -Chlordane	69	72	79	65	74	67	70			71	5	7	
<i>trans</i> -Nonachlor	81	82	88	76	89	88	85			84	5	6	
Dieldrin	85	95	103	86	96	88	102			94	7	8	
<i>p,p'</i> -DDE	110	113	110	89	93	84	111			101	12	12	
<i>o,p'</i> -DDD	85	89	94	80	89	85	100			89	7	7	
Endrin	106	111	117	115	110	102	113			111	5	5	
<i>cis</i> -Nonachlor	87	84	100	80	97	100	82			90	9	10	
<i>p,p'</i> -DDD	97	102	106	90	100	87	107			98	8	8	
<i>o,p'</i> -DDT	49	46	51	38	41	38	47			44	5	12	
<i>p,p'</i> -DDT	97	108	113	99	104	98	115			105	7	7	
<i>o,p'</i> -Methoxychlor	147	151	159	134	149	147	155			149	8	5	
<i>p,p'</i> -Methoxychlor	114	116	122	106	118	112	115			115	5	4	
Mirex	117	110	114	100	109	112	107			110	5	5	

Table 10. Percent recovery of 2.5-microgram-per-kilogram reagent spike

[Std. dev., standard deviation; RSD, relative standard deviation; NS, not spiked, standards not available; ND, not detected]

Compound	Recovery of individual compounds, in percent							Mean	Std. dev.	RSD (percent)
	A	B	C	D	E	F	G			
<i>alpha</i> -HCH	48	54	44	49	45	50	44	48	3	7
Hexachlorobenzene	84	77	59	71	67	70	62	70	9	12
Pentachloroanisole	71	75	56	69	64	69	67	67	6	9
<i>beta</i> -HCH	61	80	73	73	68	75	70	71	6	8
<i>gamma</i> -HCH	69	60	56	55	50	56	52	57	6	11
<i>delta</i> -HCH	70	102	96	96	84	95	94	91	11	12
Heptachlor	71	67	74	74	59	66	66	68	5	8
Aldrin	56	56	55	56	50	53	52	54	2	4
DCPA	87	100	92	94	93	100	93	94	5	5
Heptachlor epoxide	122	92	86	88	79	88	89	92	14	15
Oxychlorthane	NS	NS	NS	NS	NS	NS	NS			
<i>trans</i> -Chlordane	73	87	83	86	78	88	91	84	6	7
<i>o,p'</i> -DDE	NS	NS	NS	NS	NS	NS	NS			
<i>cis</i> -Chlordane	68	88	82	82	80	85	84	81	6	8
<i>trans</i> -Nonachlor	79	89	83	87	78	89	93	85	6	7
Dieldrin	58	77	73	72	59	65	62	67	7	11
<i>p,p'</i> -DDE	64	64	64	65	62	64	65	64	1	2
<i>o,p'</i> -DDD	60	74	67	67	65	71	66	67	4	6
Endrin	82	95	92	87	83	93	94	89	5	6
<i>cis</i> -Nonachlor	77	92	88	88	83	92	97	88	7	7
<i>p,p'</i> -DDD	76	73	65	68	73	81	126	80	21	26
<i>o,p'</i> -DDT	52	77	61	59	56	65	48	60	9	16
<i>p,p'</i> -DDT*	67	117	109	85	82	93	ND	92	18	20
<i>o,p'</i> -Methoxychlor	165	182	164	156	162	174	148	164	11	7
<i>p,p'</i> -Methoxychlor	216	241	238	196	203	217	184	214	21	10
Mirex	107	115	115	117	107	115	122	114	5	5

*n=6.

Table 11. Percent recovery of 40-microgram-per-kilogram reagent spike

[Std. dev., standard deviation; RSD, relative standard deviation]

Compound	Recovery of individual compounds, in percent					Mean	Std. dev.	RSD (percent)
	A	B	C	D	E			
<i>alpha</i> -HCH	86	82	79	87	84	84	3	4
Hexachlorobenzene	110	116	95	111	108	108	8	7
Pentachloroanisole	88	91	86	92	89	89	2	3
<i>beta</i> -HCH	94	88	85	92	90	90	4	4
<i>gamma</i> -HCH	91	80	89	77	84	84	6	7
<i>delta</i> -HCH	97	90	107	92	97	97	7	7
Heptachlor	84	81	92	67	81	81	9	11
Aldrin	89	87	99	67	86	86	12	14
DCPA	94	84	95	85	89	89	5	6
Heptachlor epoxide	98	86	95	63	86	86	14	16
Oxychlorthane	93	84	83	81	85	85	5	5
<i>trans</i> -Chlordane	94	89	99	81	91	91	6	7
<i>o,p'</i> -DDE	57	50	60	37	51	51	9	18
<i>cis</i> -Chlordane	94	89	97	81	90	90	6	7
<i>trans</i> -Nonachlor	89	85	92	80	87	87	5	5
Dieldrin	92	88	99	91	93	93	4	4
<i>p,p'</i> -DDE	90	100	103	79	93	93	9	10
<i>o,p'</i> -DDD	88	83	89	91	88	88	3	3
Endrin	93	89	95	81	90	90	5	6
<i>cis</i> -Nonachlor	95	90	102	81	92	92	8	8
<i>p,p'</i> -DDD	84	88	98	92	90	90	5	6
<i>o,p'</i> -DDT	64	74	70	67	69	69	4	5
<i>p,p'</i> -DDT	108	110	111	81	102	102	13	12
<i>o,p'</i> -Methoxychlor	87	90	92	68	84	84	9	11
<i>p,p'</i> -Methoxychlor	112	115	107	83	104	104	12	12
Mirex	94	94	106	74	92	92	11	12

Table 12. Percent recovery from National Institute of Standards and Technology
Standard Reference Material 1588 cod liver oil

[Std. dev., standard deviation; RSD, relative standard deviation]

Compound	Recovery of individual compounds, in percent							Mean	Std. dev.	RSD (percent)
	A	B	C	D	E	F	G			
Hexachlorobenzene	106	90	105	96	98	109	96	100	7	7
<i>alpha</i> -HCH	52	47	50	42	51	55	48	49	4	9
<i>trans</i> -Chlordane	180	166	115	104	109	122	119	131	30	23
<i>cis</i> -Chlordane	109	99	98	92	99	113	90	100	8	8
<i>trans</i> -Nonachlor	101	94	102	96	101	105	103	100	4	4
Dieldrin	73	70	76	70	81	71	74	74	4	5
<i>p,p'</i> -DDD	135	115	109	42	139	185	162	127	46	36
<i>p,p'</i> -DDE	88	73	83	72	69	90	79	79	8	11
<i>o,p'</i> -DDT	93	85	96	82	51	69	84	80	15	19
<i>p,p'</i> -DDT	81	77	97	65	74	84	79	80	10	12

Table 13a. Results of U.S. Fish and Wildlife round-robin sample 2

[Concentration units in micrograms per kilogram wet weight; NWQL, National Water Quality Laboratory; *, abbreviations of other laboratories that participated in the round-robin study; Std. dev., standard deviation; RSD, relative standard deviation]

Compound	NWQL1	NWQL2	MSCL*	GERG1*	GERG2*	MAZL1*	MAZL2*	PACFI*	PACF2*	PACF3*	Mean	Std. dev.	RSD (percent)
<i>alpha</i> -HCH	48	53	97	130	124	5	114	100	80	100	85	39	46
Hexachlorobenzene	90	51	65	40	91	100	97	72	60	79	75	20	27
<i>gamma</i> -HCH	68	66	93	110	124	15	85	96	78	97	83	30	36
Heptachlor epoxide	70	56	86	80	101	77	69	80	64	83	77	13	16
Oxychlorthane	84	68	88	90	106	89	64	83	64	84	82	13	16
<i>trans</i> -Chlordane	89	74	85	110	126	96	103	92	77	94	95	15	16
<i>o,p'</i> -DDE	81	81	80	100	115	75	99	44	56	85	82	21	25
<i>cis</i> -Chlordane	104	84	79	100	115	87	80	83	68	82	88	14	16
<i>trans</i> -Nonachlor	102	84	100	100	125	100	151	91	78	94	103	21	21
Dieldrin	101	84	90	120	97	100	118	85	80	93	97	14	14
<i>p,p'</i> -DDE	168	130	110	170	125	140	133	190	110	110	139	28	20
<i>o,p'</i> -DDD	120	84	120	40	106	99	110	100	78	100	96	24	25
Endrin	110	94	97	90	104	110	89	88	83	96	96	9	10
<i>p,p'</i> -DDD	120	99	120	160	122	90	127	110	86	110	114	21	19
<i>o,p'</i> -DDT	110	92	120	160	124	83	115	130	97	130	116	22	19
<i>p,p'</i> -DDT	120	100	98	140	116	100	159	140	110	140	122	21	17
Mirex	87	83	90	120	100	96	111	69	62	67	89	19	22
Total PCB	450	350	370	520	672	390	480	580	590	530	493	105	21
Toxaphene	400	320	490	25	246	290	530	560	490	590	394	176	45
Lipid, in percent	3.3	3.5	2.4	2.9	3.2	3.5	14.6	2.0	1.7	1.6	3.9	3.8	99.7

Table 13b. Results of U.S. Fish and Wildlife round-robin sample 12

[Concentration units in micrograms per kilogram wet weight; NWQL, National Water Quality Laboratory; *, abbreviations of other laboratories that participated in the round-robin study; Std. dev., standard deviation; RSD, relative standard deviation]

Compound	NWQL1	NWQL2	MSCL*	GERG1*	GERG2*	MAZL1*	MAZL2*	PACF1*	PACF2*	PACF3*	Mean	Std. dev.	RSD (percent)
<i>alpha</i> -HCH	45	75	97	130	129	15	132	85	96	87	89	38	42
Hexachlorobenzene	51	47	65	40	127	111	70	75	72	73	73	27	37
<i>gamma</i> -HCH	62	68	93	100	128	32	87	84	91	77	82	25	31
Heptachlor epoxide	66	76	86	90	111	73	72	70	86	75	81	13	16
Oxychlordane	78	88	87	100	106	88	66	71	80	84	85	12	14
<i>trans</i> -Chlordane	84	98	87	110	129	94	115	79	88	88	97	16	16
<i>o,p'</i> -DDE	76	85	82	110	111	76	107	44	56	85	83	22	27
<i>cis</i> -Chlordane	100	110	81	100	119	85	77	69	78	78	90	16	18
<i>trans</i> -Nonachlor	98	110	100	110	128	110	157	79	89	97	108	22	20
Dieldrin	100	62	93	120	98	98	138	85	91	61	95	23	25
<i>p,p'</i> -DDE	130	130	110	160	126	140	122	100	110	100	123	19	15
<i>o,p'</i> -DDD	91	120	120	30	107	99	113	91	100	97	97	26	27
Endrin	110	128	100	90	105	110	89	90	97	69	99	16	16
<i>p,p'</i> -DDD	110	130	130	160	126	110	128	94	100	100	119	20	17
<i>o,p'</i> -DDT	110	120	130	160	125	88	119	110	120	130	121	18	15
<i>p,p'</i> -DDT	110	130	130	150	123	110	151	120	120	150	129	16	12
Mirex	82	89	91	120	102	110	103	76	75	72	92	16	18
Total PCB	350	310	360	530	725	400	440	500	530	520	467	122	26
Toxaphene	370	420	550	25	267	270	460	490	550	600	400	174	44
Lipid, in percent	3.4	3.5	2.4	1.2	7.6	3.6	13.5	2.1	1.9	1.7	4.1	3.8	92.3

Table 14. Results of U.S. Environmental Protection Agency quality-control samples

[µg/kg, micrograms per kilogram; Std. dev., standard deviation; RSD, relative standard deviation]

Compound	Accepted value	Concentration units (µg/kg wet weight)				Mean	Std. dev.	RSD (percent)
		A	B	C	D			
<i>cis</i> -Chlordane	20	17	16	17	18	17	0.8	4.8
<i>trans</i> -Chlordane	17	12	14	15	15	14	1.4	10
<i>cis</i> -Nonachlor	9	11	10	11	11	11	.5	4.7
<i>trans</i> -Nonachlor	28	22	21	23	22	22	.8	3.7
Oxychlordane	3	3	4	3	3	3	.2	7.4
Total chlordane	78	65	65	69	69	67	2.6	3.9
Acceptable range	60-96							
<i>p,p'</i> -DDD	8	11	12	12	12	12	.5	4.3
<i>p,p'</i> -DDE	44	45	42	46	45	45	1.7	3.9
<i>p,p'</i> -DDT	0	0	0	0	0	0	0	
Total DDX	52	56	54	58	57	56	1.7	3.0
Acceptable range	32-72							
Lipid, in percent	3	6	4	5	4	5	1	20
Acceptable range	2-4							

Table 15. Method detection limits determined with reagent spike

[Std. dev., standard deviation; RSD, relative standard deviation; *n*, number of replicates; µg/kg, micrograms per kilogram; MDL, method detection limit; ND, not determined]

Compound	Mean concentration (µg/kg)	Std. dev. (µg/kg)	RSD (percent)	<i>n</i>	Amount spiked (µg/kg)	<i>t</i> -value	MDL (µg/kg)
<i>alpha</i> -HCH	1.19	0.09	7.18	7	2.5	3.1427	0.3
Hexachlorobenzene	1.75	.21	12.2	7	2.5	3.1427	.7
Pentachloroanisole	1.68	.15	8.95	7	2.5	3.1427	.5
<i>beta</i> -HCH	1.78	.15	8.20	7	2.5	3.1427	.5
<i>gamma</i> -HCH	1.42	.15	10.8	7	2.5	3.1427	.5
<i>delta</i> -HCH	2.28	.26	11.6	7	2.5	3.1427	.8
Heptachlor	1.70	.13	7.81	7	2.5	3.1427	.4
Aldrin	1.35	.05	3.99	7	2.5	3.1427	.2
DCPA	2.35	.11	4.88	7	2.5	3.1427	.4
Heptachlor epoxide	2.30	.35	15.2	7	2.5	3.1427	1.1
Oxychlordane	ND			7	2.5	3.1427	ND
<i>trans</i> -Chlordane	2.09	.16	7.48	7	2.5	3.1427	.5
<i>o,p'</i> -DDE	ND			7	2.5	3.1427	ND
<i>cis</i> -Chlordane	2.03	.16	7.64	7	2.5	3.1427	.5
<i>trans</i> -Nonachlor	2.14	.14	6.55	7	2.5	3.1427	.4
Dieldrin	1.67	.19	11.2	7	2.5	3.1427	.6
<i>p,p'</i> -DDE	1.60	.02	1.56	7	2.5	3.1427	ND
<i>o,p'</i> -DDD	1.68	.11	6.30	7	2.5	3.1427	.3
Endrin	2.24	.13	6.02	7	2.5	3.1427	.4
<i>cis</i> -Nonachlor	2.20	.16	7.45	7	2.5	3.1427	.5
<i>p,p'</i> -DDD	2.01	.52	25.9	7	2.5	3.1427	1.6
<i>o,p'</i> -DDT	1.49	.24	15.9	7	2.5	3.1427	.7
<i>p,p'</i> -DDT	1.98	.97	49	7	2.5	3.1427	3.0
<i>o,p'</i> -Methoxychlor	4.11	.28	6.79	7	2.5	3.1427	.9
<i>p,p'</i> -Methoxychlor	5.34	.53	9.86	7	2.5	3.1427	1.7
Mirex	2.85	.13	4.72	7	2.5	3.1427	.4

Table 16. Method detection limits determined with homogenized fish tissue

[Std. dev., standard deviation; RSD, relative standard deviation; *n*, number of replicates; µg/kg, micrograms per kilogram; MDL, method detection limit]

Compound	Mean concentration (µg/kg)	Std. dev. (µg/kg)	RSD (percent)	<i>n</i>	Amount spiked (µg/kg)	<i>t</i> -value	MDL (µg/kg)
<i>alpha</i> -HCH	2.13	0.1	4.87	7	2.0	3.1427	0.31
Hexachlorobenzene	1.79	.23	13.1	7	2.0	3.1427	.74
Pentachloroanisole	2.11	.25	12	7	2.0	3.1427	.76
<i>beta</i> -HCH	2.15	.42	19.6	7	2.0	3.1427	1.26
<i>gamma</i> -HCH	2.06	.28	13.7	7	2.0	3.1427	.85
<i>delta</i> -HCH	1.95	.19	9.5	7	2.0	3.1427	.56
Heptachlor	1.59	.18	11.2	7	2.0	3.1427	.56
Aldrin	1.73	.15	8.65	7	2.0	3.1427	.47
DCPA	1.75	.17	9.66	7	2.0	3.1427	.51
Heptachlor epoxide	2.01	.22	10.8	7	2.0	3.1427	.66
Oxychlorthane	2.05	.48	23.5	7	2.0	3.1427	1.44
<i>trans</i> -Chlordane	1.94	.29	15.1	7	2.0	3.1427	.88
<i>o,p'</i> -DDE	1.8	.36	20.1	7	2.0	3.1427	1.09
<i>cis</i> -Chlordane	2.35	.24	10.4	7	2.0	3.1427	.73
<i>trans</i> -Nonachlor	2.18	.16	7.27	7	2.0	3.1427	.47
Dieldrin	2.18	.31	14.1	7	2.0	3.1427	.92
<i>p,p'</i> -DDE	2.06	.32	15.6	7	2.0	3.1427	1.01
<i>o,p,p'</i> -DDD	2.08	.22	10.5	7	2.0	3.1427	.66
Endrin	2.1	.2	9.52	7	2.0	3.1427	.6
<i>cis</i> -Nonachlor	1.9	.32	16.6	7	2.0	3.1427	.95
<i>p,p'</i> -DDD	2.09	.35	16.7	7	2.0	3.1427	1.04
<i>o,p,p'</i> -DDT	2.23	.29	12.9	7	2.0	3.1427	.86
<i>p,p'</i> -DDT	2.38	.22	9.21	7	2.0	3.1427	.66
<i>o,p'</i> -Methoxychlor	1.81	.22	12	7	2.0	3.1427	.66
<i>p,p'</i> -Methoxychlor	1.83	.18	9.6	7	2.0	3.1427	.53
Mirex	1.96	.37	19.1	7	2.0	3.1427	1.17